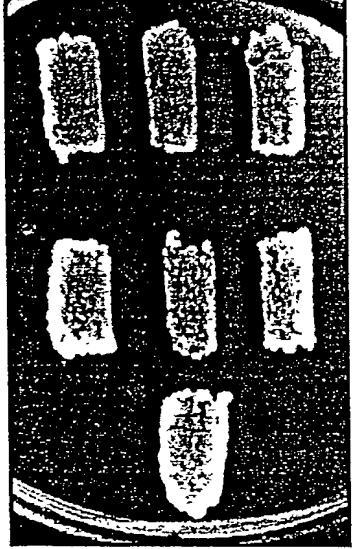


A

Fig 1

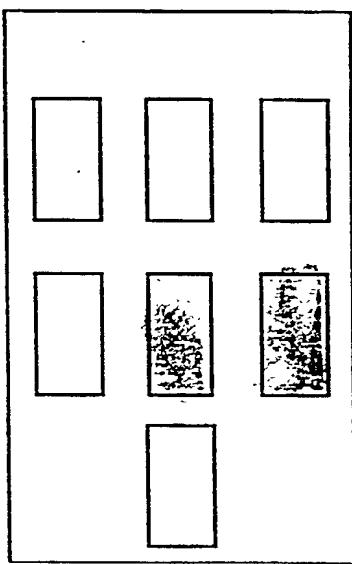
PH Domain + PIF	...
Δ PH-PDK1 + PIF	...
PDK1 + PIF	...
---	---



C



D



1/22

Region A

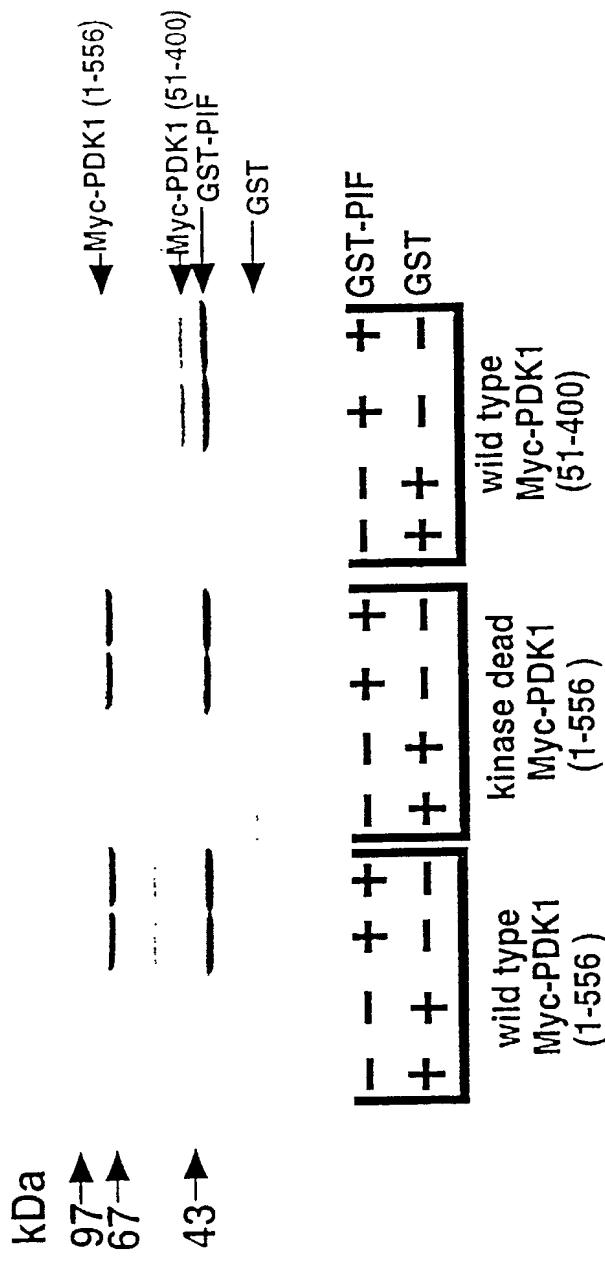
EDVKKHPFFRLIDWALMDDKKVVKPPFPIPTIRGREDVSNEDFTEAFTSEAPILTPP--
908 PRK2 908 EDVKKHPFFRLIDWALMDDKKVVKPPFPIPTIRGREDVSNEDFTEAFTSEAPILTPP--
403 PRK1 403 EDVKKHPFFRLIDWALMDDKKVVKPPFPIPTIRGREDVSNEDFTEAFTSEAPILTPP--
400 PKB α 400 KEMMQHRRFFAGIVMQLHUYEKKKSSPPEKPOVTSSETDTRYFEDFEFTSOMITTPDQQDSMEECVDSERRP--
321 p70S6k 321 GEVQAHPEFFRHIINMEELLIAKVEPPPKPLQSEEDVSVIEDSKFTRTTPDDEP--
347 SGK 347 MEFKSHMFFSSLINAKKKITPPENPNVSGPNKSHHEDPEFTEEPVPNLSICKSPS
510 PKC ζ 510 SDIKSHAFERSIDMDLLEKQALPPFQQPQITDDYGDADNFDTKFTSEEPVQLTPE--
589 PKC α 589 RDVREHAFERRIDMEKLENTEQOPPKPKYCG-KGAENFDKFFTRGQEVLTPE--
290 PKA β 290 SDIKTHKQFATTDMIAVYQKVEAPPKFGRSQGDTSNFDDYEEEYIYQ--

Region B

PRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--
984 PDK2 984 PRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--
479 PRK1 479 PRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--
480 PKB α 480 PRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--
395 p70S6k 395 PRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--
428 SGK 428 PRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--
585 PKC ζ 585 PRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--
663 PKC α 663 PRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--
351 PKA β 351 PRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--FPRILSEEEOEN--

PDK2 MOTIF

A glutathione-Sephadex "Pull Down"



2/22

Fig 2 (page 1 of 3)

Myc antibody immunoprecipitate

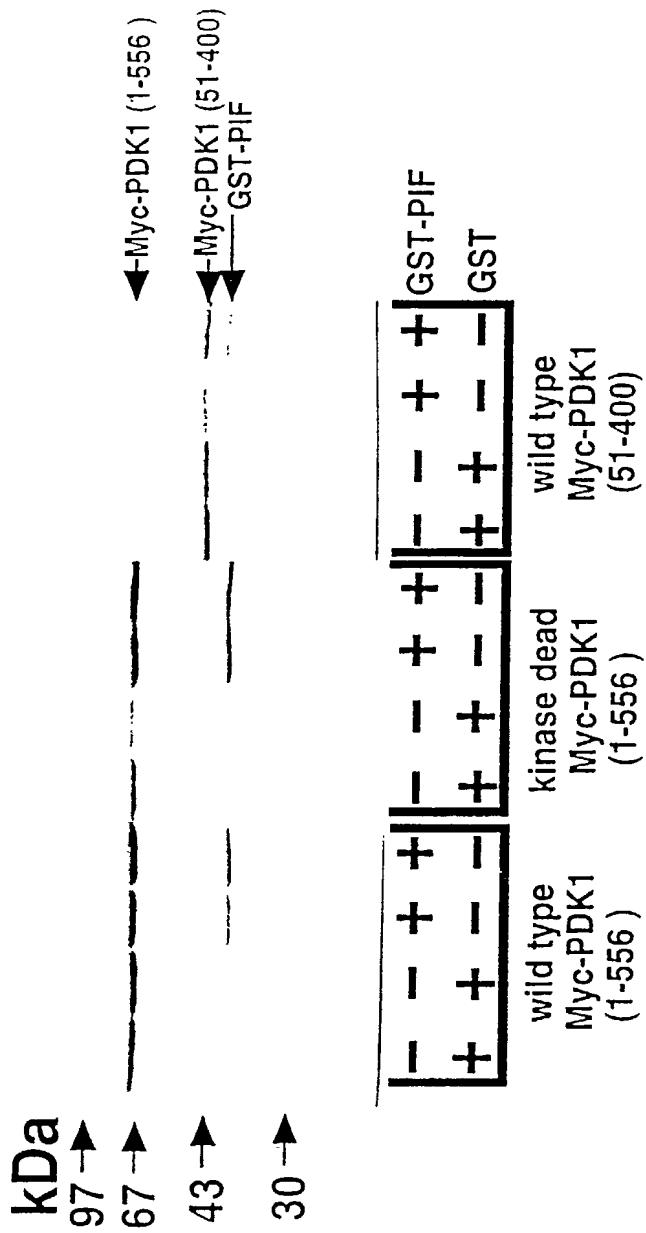
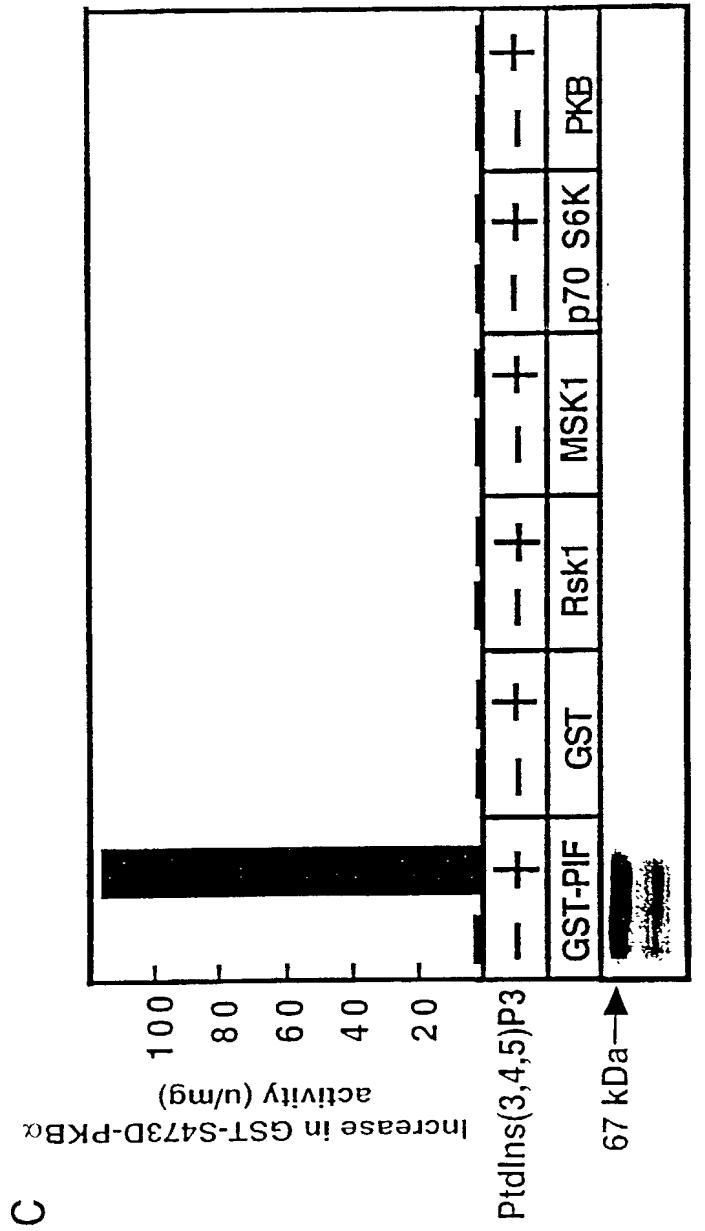
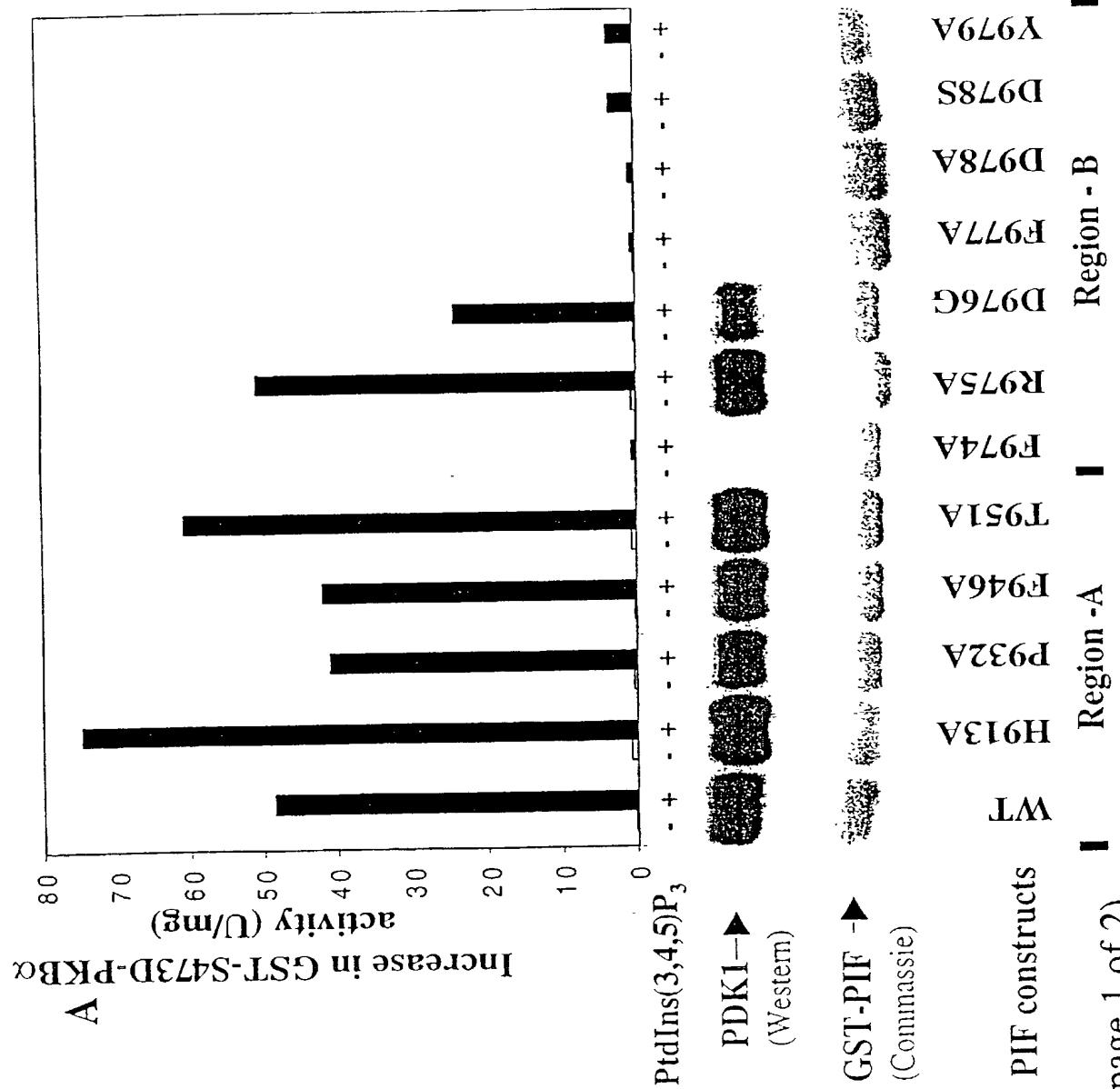


Fig 2 (page 2 of 3)



Western blot for endogenous PDK1

Fig 2 (page 3 of 3)



5/22

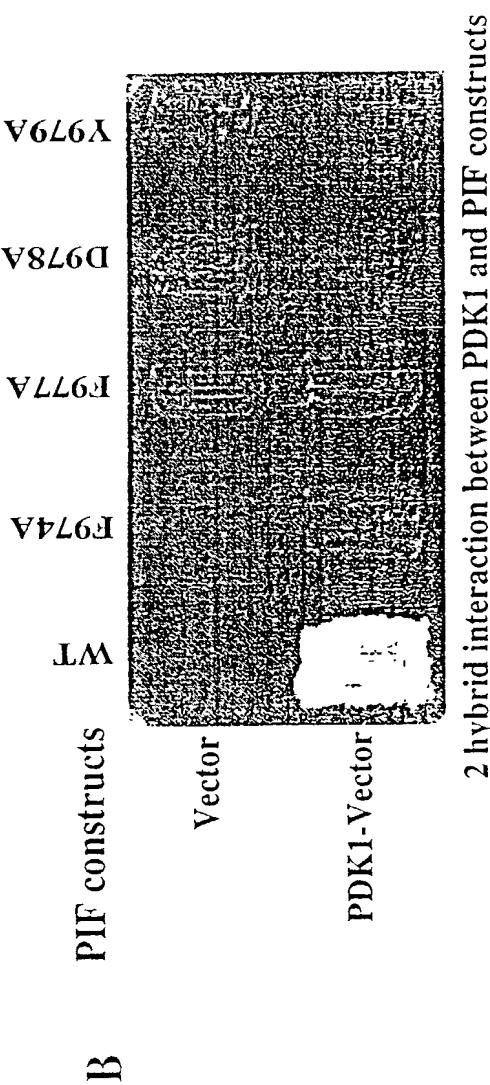


Fig 3 (page 2 of 2)

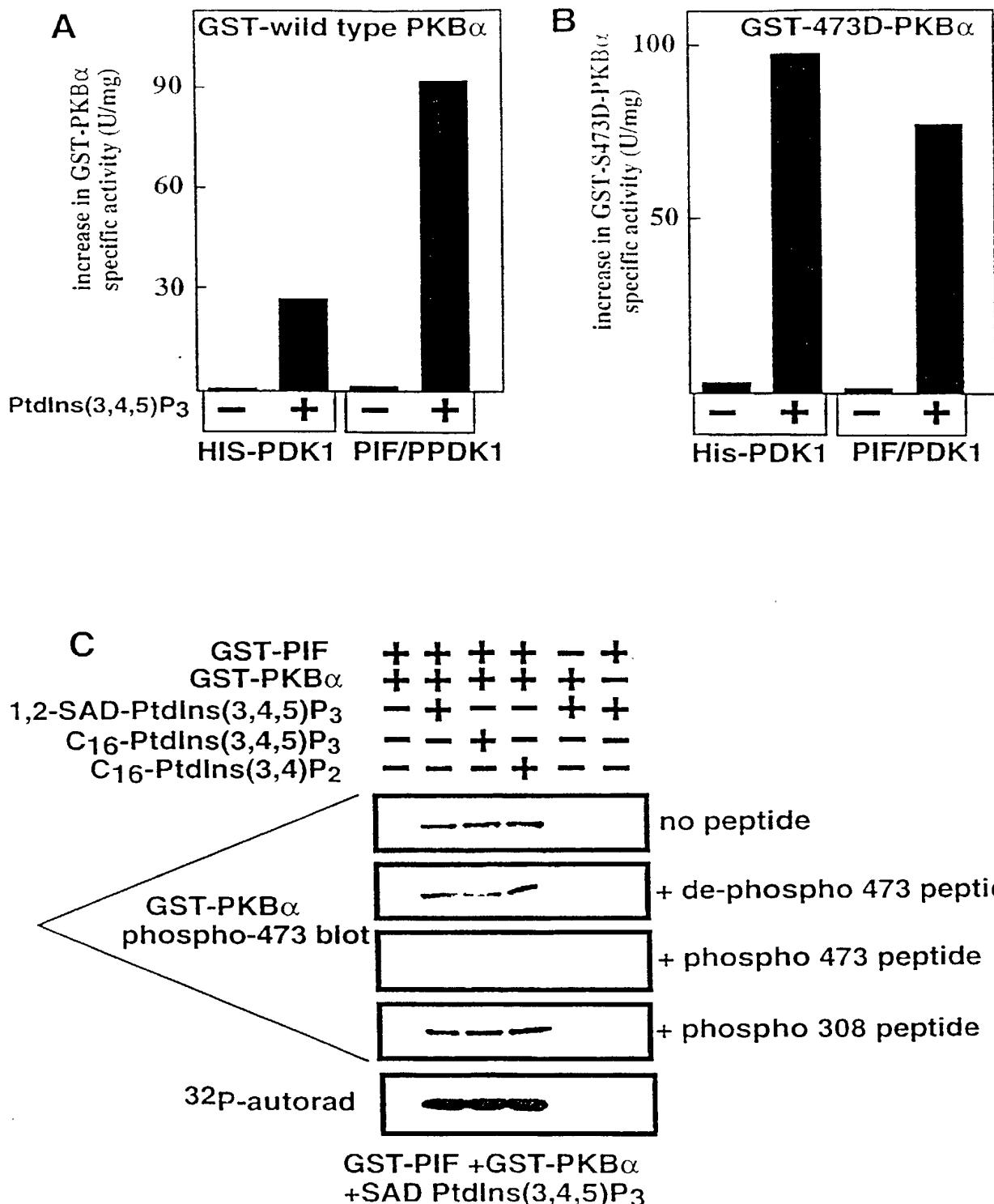


Fig 4 (page 1 of 2)

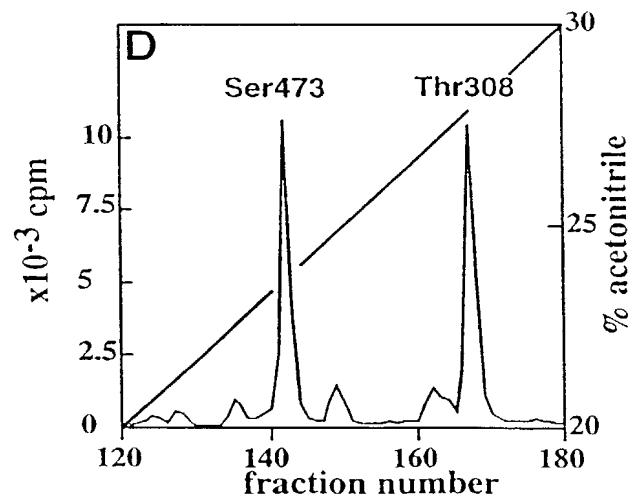


Fig 4 (page 2 of 2)

8/22

A

	Control	Non specific antibody	PDK1 protein antibody	PDK1 peptide antibody	PDK1 peptide + Peptide
1,2-SAD-PtdIns(3,4,5)P ₃	- + -	- + -	- + -	- + -	- + -
C16-PtdIns(3,4,5)P ₃	- - + -	- - + -	- - + -	- - + -	- - + -
C16-PtdIns(3,4)P ₂	- - + -	- - + -	- - + -	- - + -	- - + -

PKB α phospho-Ser473 blot in supernatant of immunoprecipitates**B**

	H913A	P932A	F946A	T951A	Q971A	F974A	R975A	D976G	F977A	D977A	D978S	Y977A
1,2-SAD-PtdIns(3,4,5)P ₃	- +	- +	- +	- +	- +	- +	- +	- +	- +	- +	- +	- +

PKB α phospho 473 blot

9/22

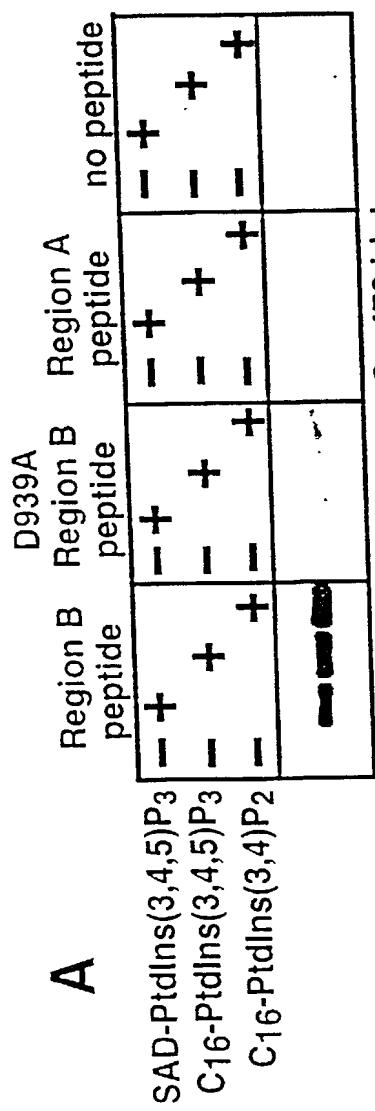
Region A

Region B

935-FRDFDY-940

PDK2 motif in PRK2

Fig 5



10/22

SUBSTITUTE SHEET (RULE 26)

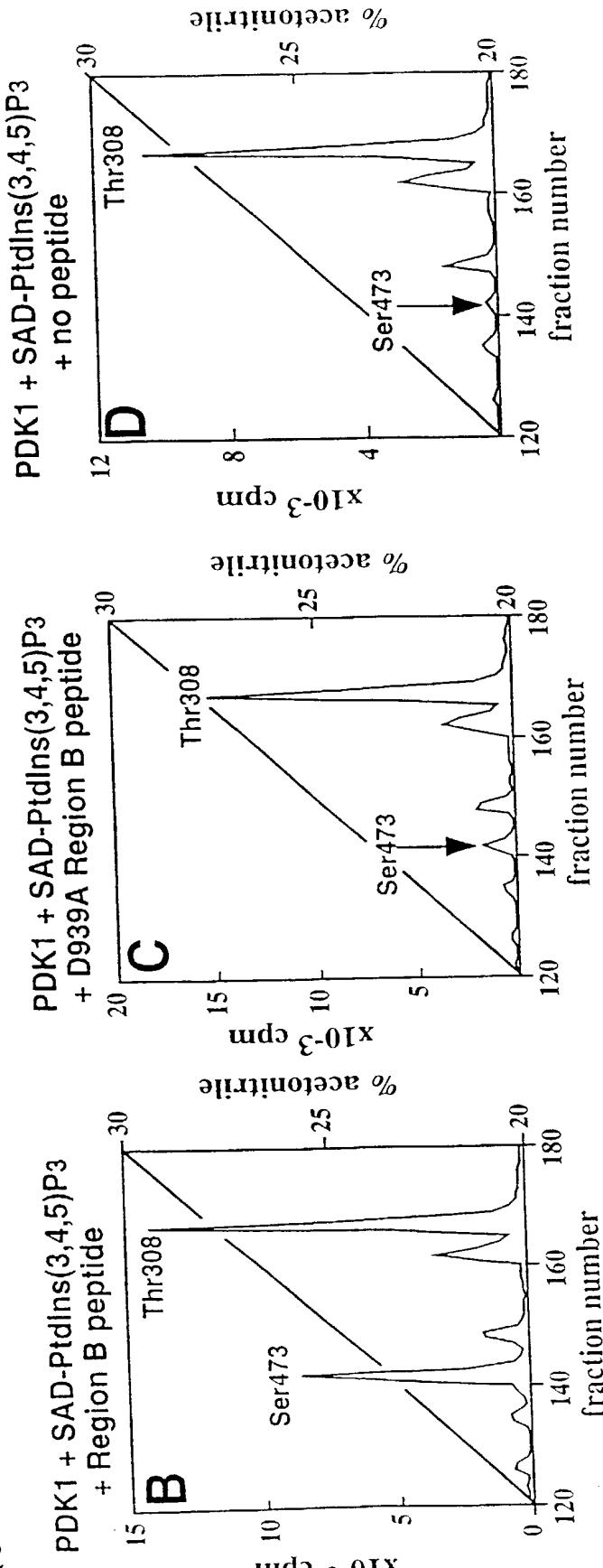


Fig 6

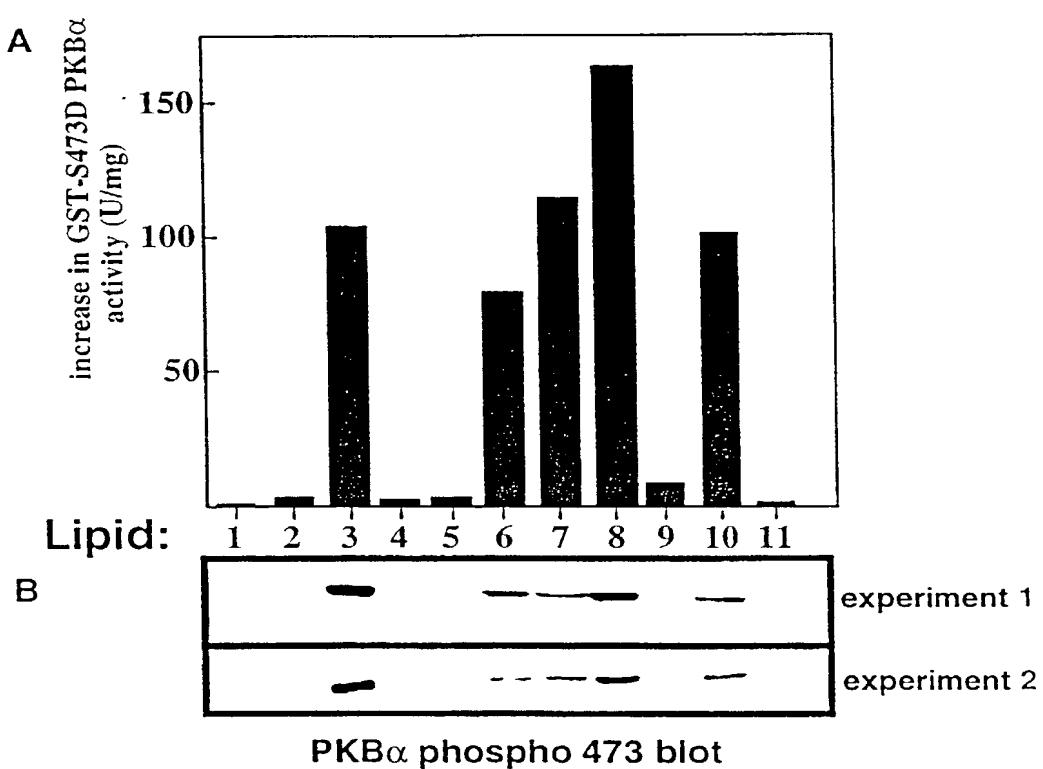


Fig 7

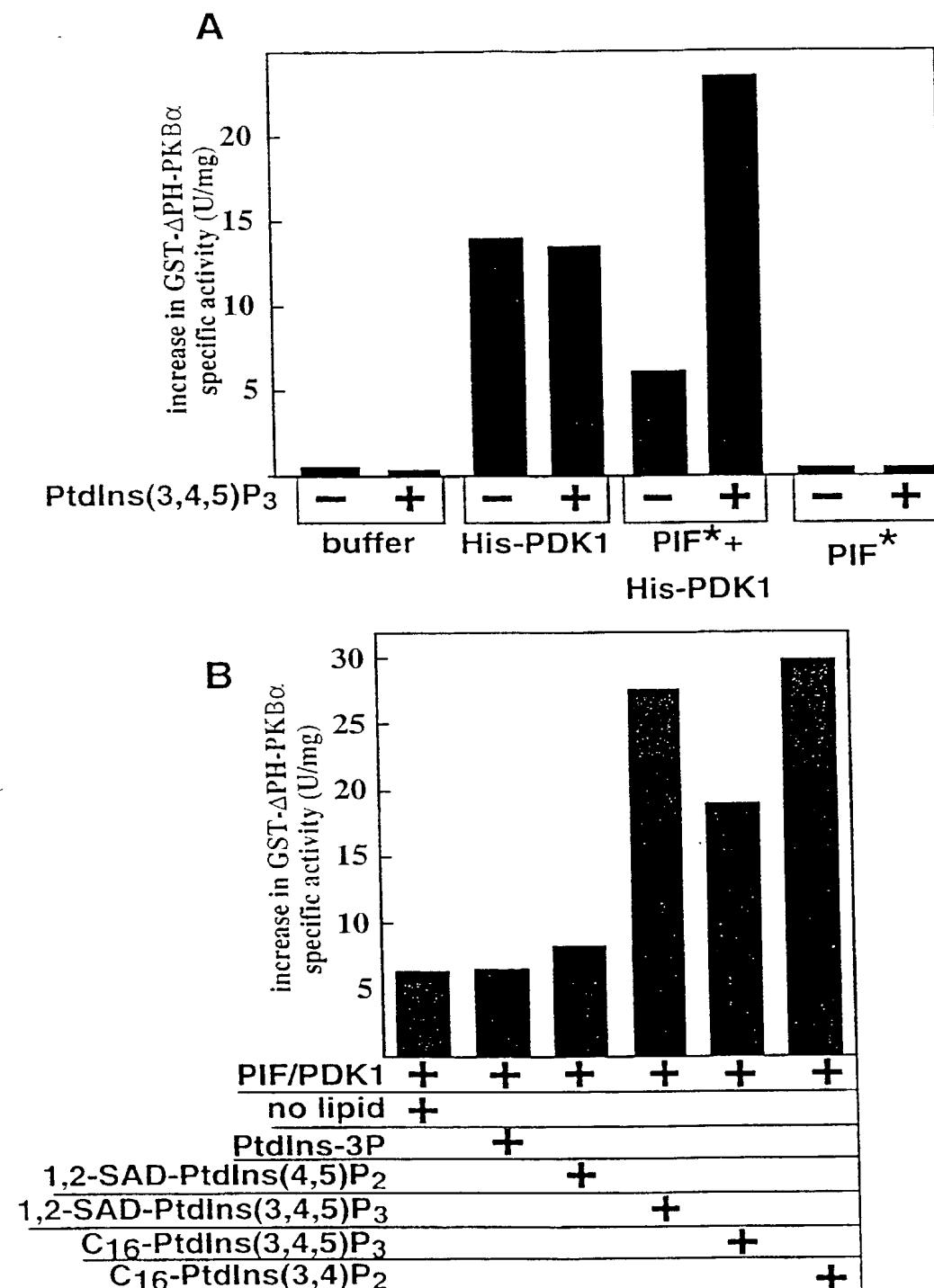


Fig 8

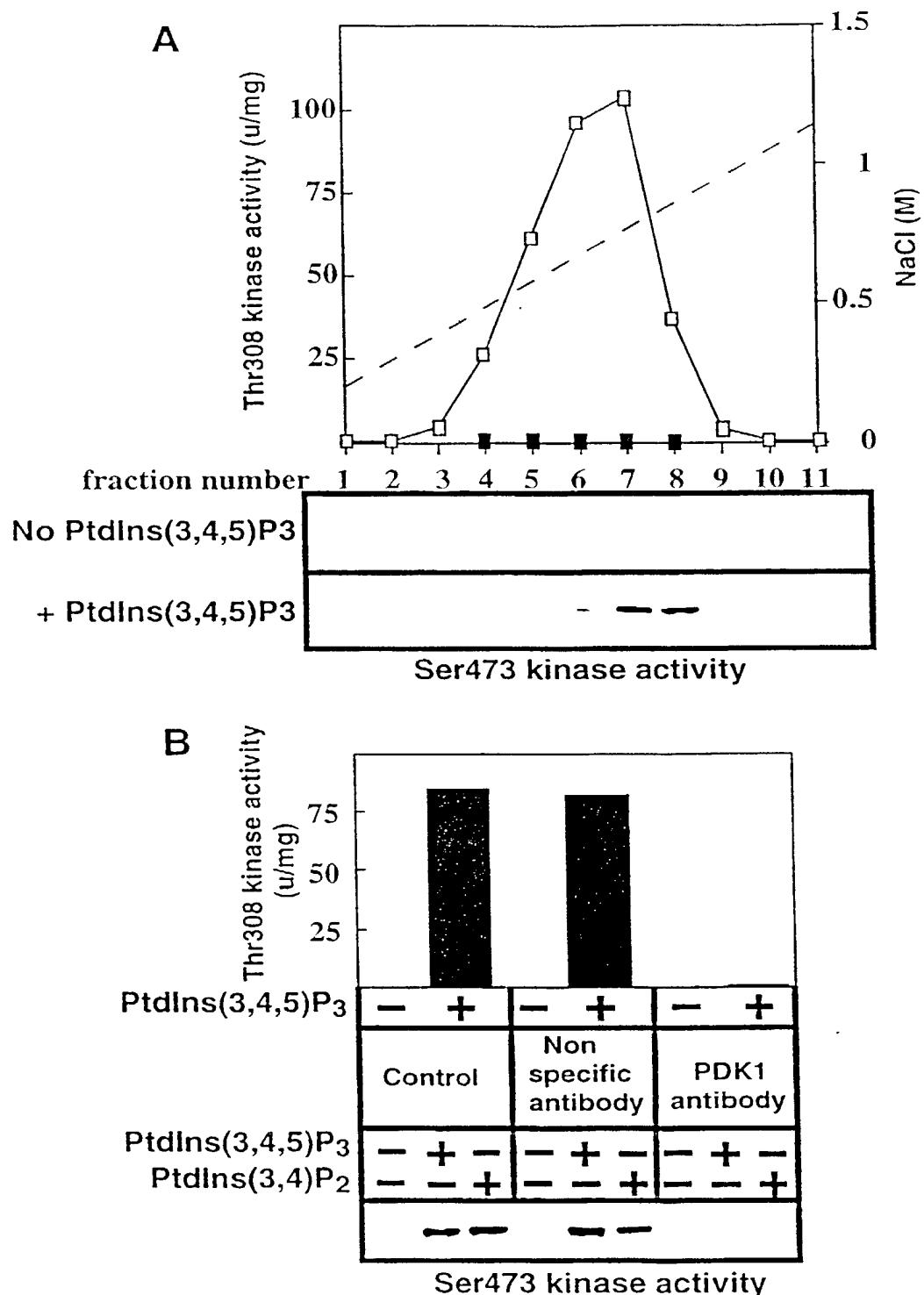


Fig 9

Fig 10

MARTTSQLYDAVPIQSSVVLCS
CPSPSMVRTQTESSTPPGIPGGSRQGPAM
DGTAAEPRPGAGSLQHAQPPPQPRKKRPEDFKFGKILGE
GEGSFSTVVLAREL
ATSREYAIKILEKRHI
I
KENKVPYVTRERDVM
SRLDHPFFVKLYFTFQDDE
KLYFGLSYAKNGELLKYIRKIGSF
DETCTRFYTAEIVSALEYLHGKGIIHR
DLKPENILLNEDMHI
QITDFGTA
KVLSPESKQARANSFVGTAQYVPELLT
EKSACKSSDWALGCIIYQLVAGLPPFRAGNEYLIFQKII
KLEYDFPEKFF
PKARDLVEKLLVLDATKRLGCEEMEGYGPLKA
HPFFESVTWENLHQQT
PPK
LTAYLPAMSEDDED
CYGNYDNLLSQFGCMQVSSSSSHSLSAS
DTGLPQRS
GSNIEQYIH
LDSNSFELDLQFSEDEK
RLLLEKQAGGNPWHQF
VENNLILK
MGPVDKRKGLFARRQ
QLLTEGPHLYVDPVN
KVLKGEIPWSQELR
PEAKN
FKTFFVHTPNRTYYL
MDPSGN
AHK
WCRK
I
QEV
WRQ
RYQ
SHPD
AAVQ

PDK1 sequence

Fig 11 (page 1 of 2)

Human PRK2 sequence information:

LOCUS 1000125 984 aa 04-FEB-1999
 DEFINITION PRK2
 ACCESSION 1000125
 PID g1000125
 DBSOURCE GENBANK: locus HSU33052, accession U33052
 KEYWORDS
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 1 (residues 1 to 984)
 REFERENCE Palmer,R.H., Ridden,J. and Parker,P.J.
 AUTHORS Palmer,R.H., Ridden,J. and Parker,P.J.
 TITLE Identification of multiple, novel, protein kinase C-related
 gene products
 JOURNAL FEBS Lett. 356 (1), 5-8 (1994)
 MEDLINE 95080426
 REFERENCE Palmer,R.H., Ridden,J. and Parker,P.J.
 AUTHORS Palmer,R.H., Ridden,J. and Parker,P.J.
 TITLE Cloning and expression patterns of two members of a novel
 protein-kinase-C-related kinase family
 JOURNAL Eur. J. Biochem. 227 (1-2), 344-351 (1995)
 MEDLINE 95154310
 REFERENCE Palmer,R.H.
 AUTHORS Palmer,R.H.
 TITLE Direct Submission
 JOURNAL Submitted (02-AUG-1995) Ruth H. Palmer, Protein
 Phosphorylation,
 ICRF, 44 Lincoln's Inn Fields, London, WC2A 3PX, UK
 COMMENT Method: conceptual translation.
 FEATURES Location/Qualifiers
 source 1..984
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 /clone_lib="cDNA library from human DX3 cell line
 (B-cell
 lineage)"
 Protein 1..984
 /product="PRK2"
 CDS 1..984
 /note="lipid-activated, protein kinase C-related,
 serine/threonine protein kinase"
 /coded_by="U33052.10..2964"

ORIGIN

1 masnpergei lltelqgdsr slpfsevsa vqkldfsdtm vqqklddkd rikreirkel
 61 kikegaenlr kvtdkksla yvdnilkksn kkleelhhkl gelnahivvs dpeditdcpr
 121 tpdtppndpr cstsnnrlka lqkqlldielk vqggaenmq mysngsskdr klhgtaqql1
 181 qdsktkiev1 rmqilqavqt nelafdnakp vispleirme elrhhfrief avaegaknvm
 241 kllgsgkvtd rkalseaqr tnessqkldi ikysieqrln evpknnoksr 111eelslva
 301 asptlspqrq mislqnqyst lskpaaltgt levrlmgcqd 1lenvpgrsk atsvalpgws
 361 psetrssfms rtsksksgss rnllktddls ndvcavlkld ntvvqgtswk pisnqswdqk
 421 ftleldrsre leisvwyrdw rslcavkflr ledfldnqrh gmclylepog tlfiaeavtffn

481 pvierrpklq rkdkifskqq gktflrapqm niniatwgrl vrraipvtvh sgtfspqapv
541 pttvpvvdrv ipqlappasd stvtkldfdl epepppappr asslgeides selrvldipg
601 qdsetvfdiq ndrnsilpks qseykpdtq sgleysgqe ledrrsqqrq cfnlqdfrc
661 avlgrghtgk vllaeykntn emfaikalkk gdvardevd slmcekrife tvnsvrhpfl
721 vnlfacfqtk ehvcfvmeya aggdlmmhih tdfvseprav fyaacvvlgl cylhehkivy
781 rdlkldnlll dtegfvkiad fglckegmgy gdrtstfcgt peflapevlt etsytravdw
841 wglgvliyem lvgespfpgd deeevfdsiv ndevryprfl steaisimrr ilrrnperrl
901 gasekdaedv kkhpffrlid wsalmdkkvk ppfiptirgr edvsnfdddef tseapiltpp
961 reprisseee qemfrdfdyi adwc

Fig 11 (page 2 of 2)

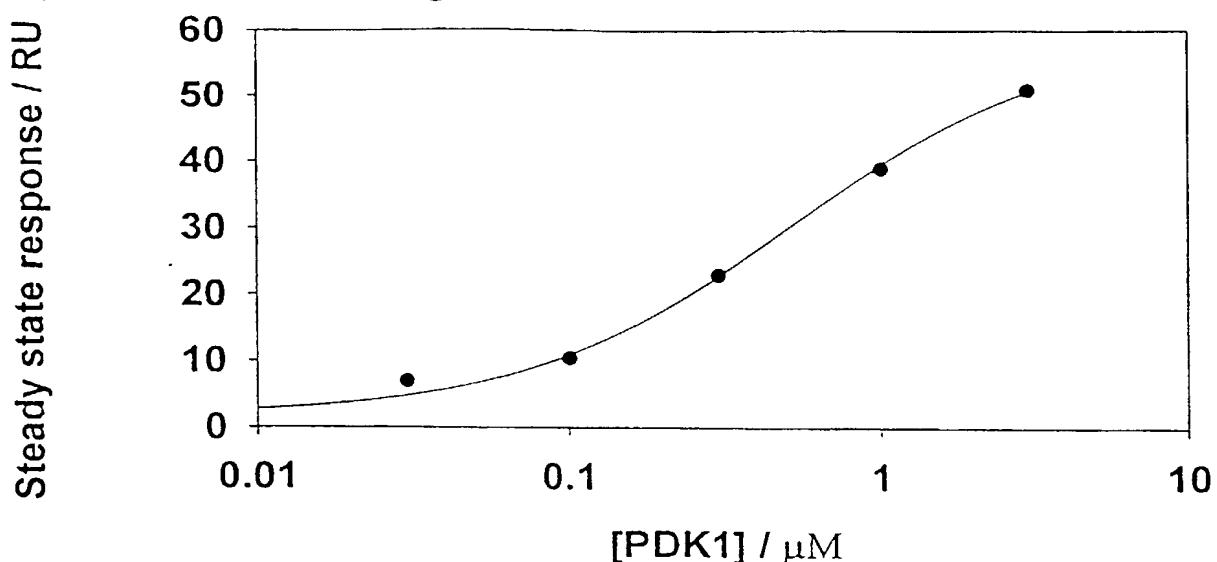
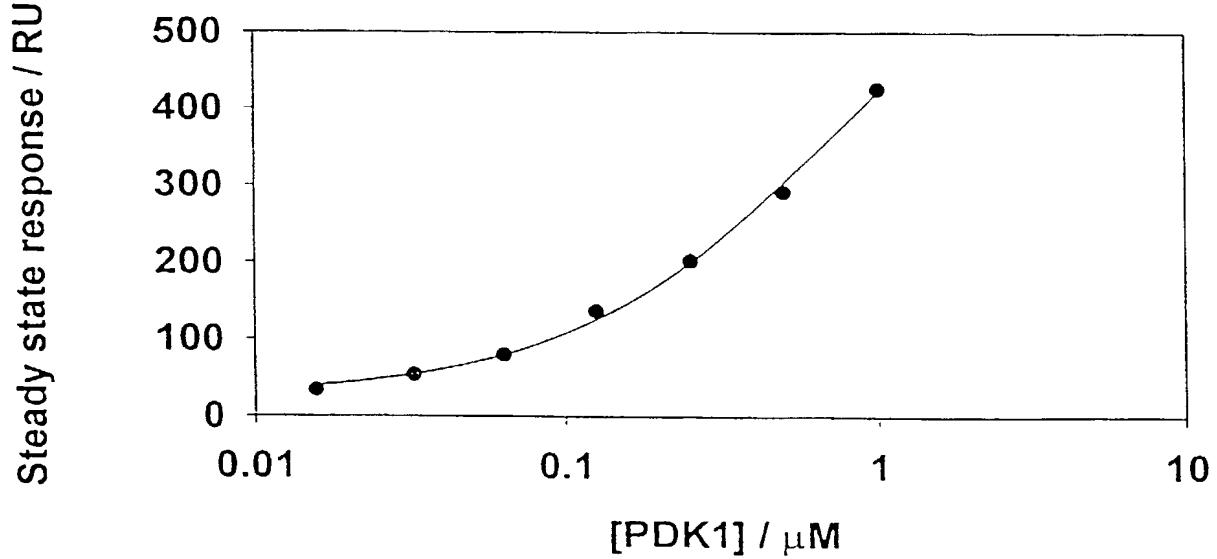
(A) HisPDK1 binding to GST-PIF**(B) HisPDK1 binding to PIF region B peptide**

Fig 12 (page 1 of 2)

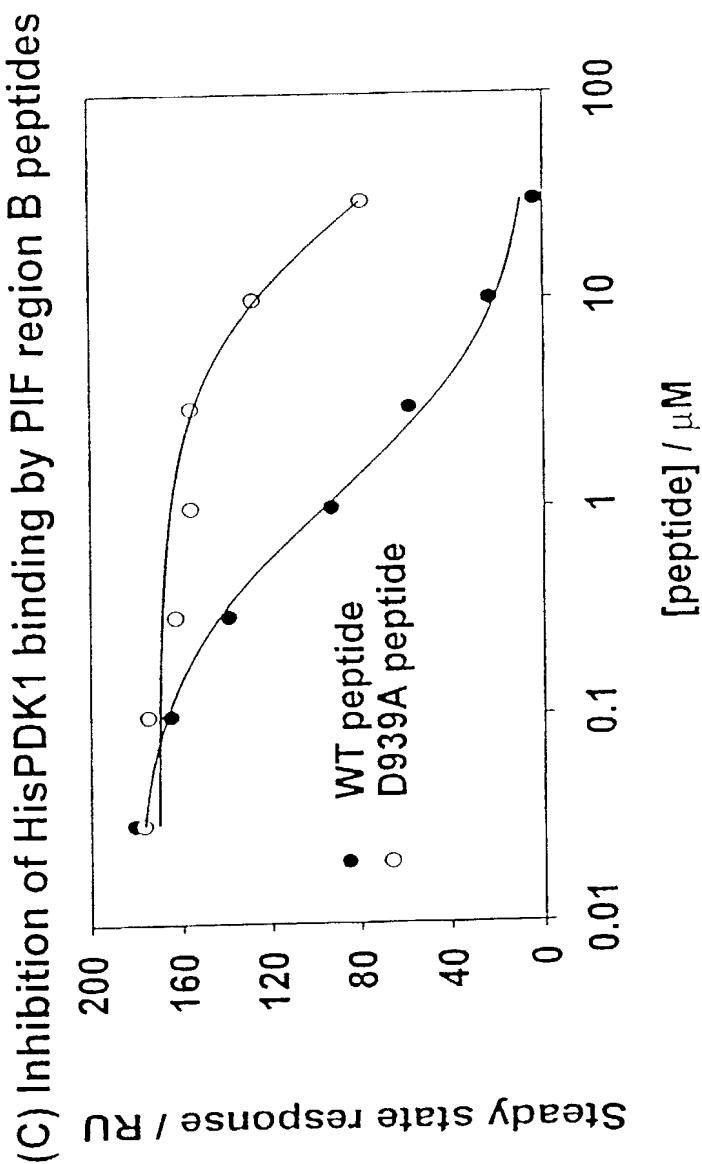


Fig 12 (page 2 of 2)

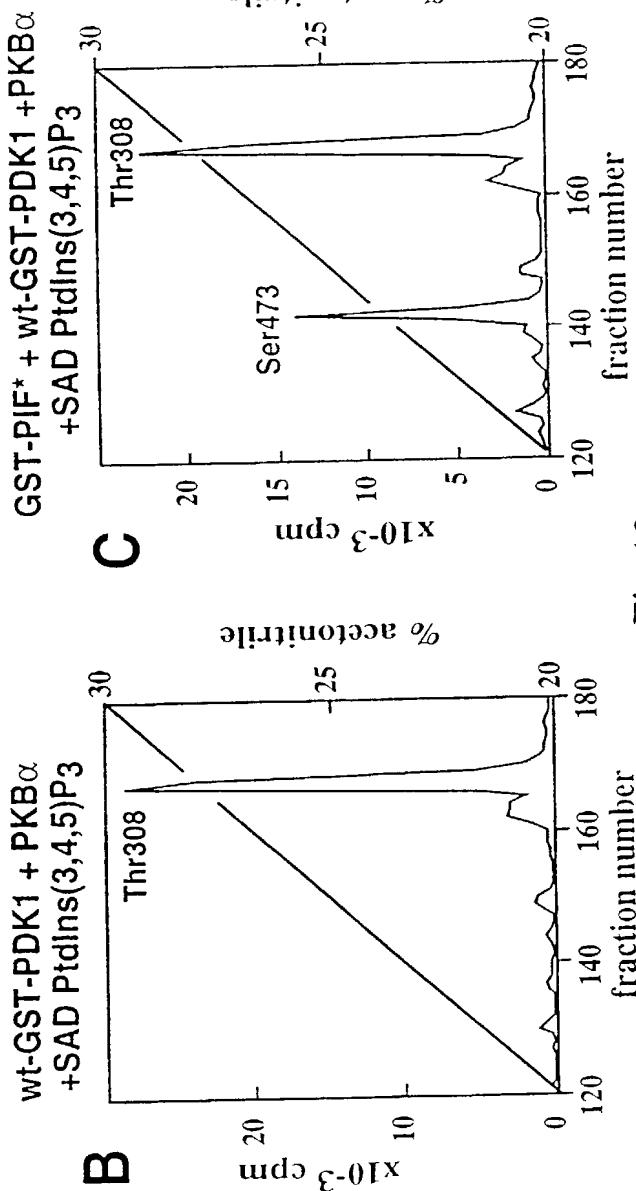
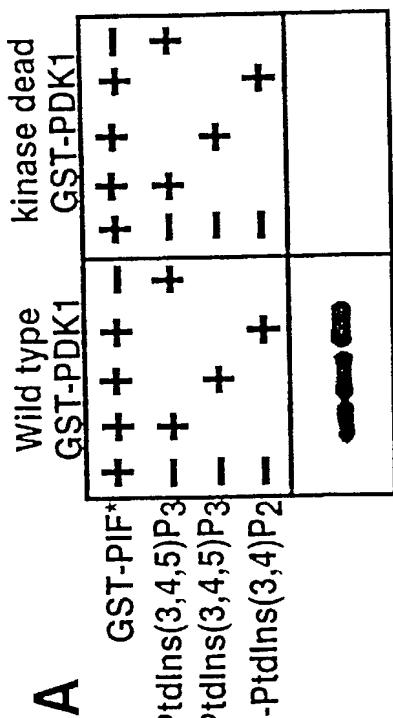


Fig 14

Human PRK1 sequence information:

LOCUS 2136028 942 aa 02-JUL-1996
 DEFINITION protein kinase PRK1 - human.
 ACCESSION 2136028
 PID g2136028
 DBSOURCE PIR: locus I53327
 summary. #length 942 #molecular-weight 103989 #checksum 8328.
 PIR dates: 02-Jul-1996 #sequence_revision 02-Jul-1996
 #text_change 02-Jul-1996.
 KEYWORDS
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Mammalia, Eutheria; Primates; Catarrhini;
 Hominidae;
 REFERENCE 1 (residues 1 to 942)
 AUTHORS Palmer, R.H., Ridden, J. and Parker, P.J.
 TITLE Cloning and expression patterns of two members of a novel
 protein-kinase-C-related kinase family
 JOURNAL Eur. J Biochem. 227 (1-2), 344-351 (1995)
 MEDLINE 95154310
 FEATURES Location/Qualifiers
 source 1..942
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 Protein 1..942
 /product="protein kinase PRK1"

ORIGIN

1 masdavqsep rswsllqelqg lagadlaapg vqqglelere rlrreirkel lkegaenlr
 61 rattdlgrsl gpvelliargs srrlldlhqq lqelhahvvl pdpaathdgp qspgaggptc
 121 satnlsrvag lekqlailelk vkqgaenmq tysngstkdr klltaqqml qdsstkidii
 181 rmqlrralqa dqlenqaapd dtqgspdlga veirieelrh hfrvehavae gaknvlrls
 241 aakapdrkav seagekltes nqklglrea lerrlgelpa dhpkgrrlre elaaassaaaf
 301 strlagpfpa thystlckpa pltgtlevrv vgrcdlpeti pwnptpsmgg pgtpdsrppf
 361 lsrparglys rsgsllsgrss lkaeaentse vstvlkldnt vvgqtswkpc gpnawdqsft
 421 lelerarele lavfwrdqrg lcalkflkle dfldnerhev qldmepqgcl vaevtfrnpv
 481 ieriprllrq kkifskqqgk afqrarqmni dvatwvrlr rlipnatgtg tfspgaspgs
 541 earttgdisv eklnlgtdsd sspqkssrpd psspsslssp iquestapelp setqetpgpa
 601 lcsplrkspf tledfkflav lgrghfgkv1 lsefrpsgel faikalkkgd ivardevesl
 661 mcekrlaav tsaghpfvn lfgcfqtpah vcfvmeysag gdlmlhihsd vsepralif
 721 sacvvlgqlf lhehkivyrd lkldnlldt egyvkiadfg lckegegmygd rtstfcgtpe
 781 flapevitdt sytravdwg lgvilyemlv gespfpgdde eevfdsivnd evryprflsa
 841 eaigimrrll rrrperrrlgs serdaedvkk qpfrrtlgw allarrlppp fvptlsgrtd
 901 vsnfddeeftg eaptlspprd arpltaaeqa afldfdfvag gc

20/22

09/937009

PCT/GB00/01004

WO 00/56864

Fig 15 (page 1 of 2)

Human PKC zeta sequence information:

LOCUS 478322 592 aa 28-FEB-1997
 DEFINITION protein kinase C (EC 2.7.1.-) zeta - human.
 ACCESSION 478322
 PID g478322
 DBSOURCE PIR: locus JN0877
 summary: #length 592 #molecular-weight 67731 #checksum 87.
 genetic: #gene GDB:PRKCZ ##cross-references GDB:128040.
 superfamily: protein kinase C zeta; protein kinase C zinc-
 binding repeat homology; protein kinase homology.
 PIR dates: 03-May-1994 #sequence_revision 03-May-1994
 #text_change 28-Feb-1997.
 KEYWORDS ATP; phorbol ester receptor; phosphotransferase;
 serine/threonine-specific protein kinase; zinc.
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
 Hominidae; Homo.
 REFERENCE 1 (residues 1 to 592)
 AUTHORS Kochs,G., Hummel,R., Meyer,D., Hug,H., Marme,D. and
 Sarre,T.F.
 TITLE Activation and substrate specificity of the human protein
 kinase C alpha and zeta isoenzymes
 JOURNAL Eur. J. Biochem. 216 (2), 597-606 (1993)
 MEDLINE 93387312
 REFERENCE 2 (residues 1 to 592)
 AUTHORS Barbee,J.L., Deutscher,S.L., Loomis,C.R. and Burns,D.J.
 TITLE The cDNA sequence encoding human protein kinase C-zeta
 JOURNAL Gene 132 (2), 305-306 (1993)
 MEDLINE 94040779
 REFERENCE 3 (residues 1 to 592)
 AUTHORS Hug, H.
 TITLE Direct Submission
 JOURNAL Submitted (??-SEP-1992) to the EMBL Data Library
 FEATURES Location/Qualifiers
 source 1..592
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 Protein 1..592
 /product="protein kinase C zeta"
 /EC_number="2.7.1.-"
 Region 131..180
 /note="protein kinase C zinc-binding repeat homology
 #label KZ1"
 /region_name="domain"
 Region 250..518
 /note="protein kinase homology #label KIN"
 /region_name="domain"
 Region 258..266
 /note="protein kinase ATP-binding motif"
 /region_name="region"
 Site 281
 /note="Lys"

09/937009

WO 00/56864

PCT/GB00/01004

/site_type="active"

ORIGIN

1 mpsrtdpkme gsggrvrlka hyggdifts vdaattfeel ceevrdmcrl hqqhpltlkw
61 vasegdptcv ssqmeleefaf rlarcrcdeg llihvfstp eqpglpcpge dksiyrrgar
121 rwrklyrang hlfqakrfnr raycqgqcsr iwglaqqyr cinckllvhk rchglvpltc
181 rkhmdsvmps qeppvddknc dadlpseetd grayisssrk hdsikddsed lkpvldgmdg
241 ikisqqlglq dfdlirvigr gtyakvlvr lkkndqiyam kvvkkelvhd dedidwvqte
301 khvfcqassn pflvglhscf qtsrlflvi eyvnngdlnf hmqrqrklpe eharfyaaei
361 cialnflher giyrdlkld nvlldadghi kldtygmcke glpgdttst fcgtptyiap
421 eilrgeeygf svdwalgv1 mfemmagrsp fdiltndpdm ntedylfqvi lekpiriprf
481 lsvkashvlk qflnkdpker lgcrpqtgfs dikshaffrs idwdllekkq alppfqpqit
541 ddygldnfdt qftsepvqlt pddedaikri dqsefegfey inpllistee sv

Fig 15 (page 2 of 2)